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### Synthesis and Antimicrobial Activity of Some 2-(Benzo[ d ]oxazol/benzo[ d ]imidazol-2-ylthio)- N -(9 H -fluoren-9-yl)acetamide Derivatives

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Published online: 30 Jan 2007.

To cite this article: Gülhan Turan-Zitouni , Zafer Asim Kaplancikli , Ahmet Özdemir , Gilbert Revial & Kiymet Güven (2007) Synthesis and Antimicrobial Activity of Some 2-(Benzo[ d ]oxazol/benzo[ d ]imidazol-2-ylthio)- N -(9 H -fluoren-9-yl)acetamide Derivatives, *Phosphorus, Sulfur, and Silicon and the Related Elements*, 182:3, 639-646, DOI: [10.1080/10426500601047016](https://doi.org/10.1080/10426500601047016)

To link to this article: <http://dx.doi.org/10.1080/10426500601047016>

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## Synthesis and Antimicrobial Activity of Some 2-(Benzo[d]oxazol/benzo[d]imidazol-2-ylthio)-N- (9H-fluoren-9-yl)acetamide Derivatives

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*Some 2-(benzo[d]oxazol/benzo[d]imidazol-2-ylthio)-N-(9H-fluoren-9-yl)acetamide derivatives were synthesized by reacting 9-(chloroacetyl amino)fluorene with benzo[d]oxazol/benzo[d]imidazol-2-thiole in acetone in the presence of  $K_2CO_3$ . Chemical structures of the compounds were elucidated by  $^1H$  NMR spectroscopy and  $FAB^+$  mass spectrometry. Their antimicrobial activities against *Micrococcus luteus* (NRRL B-4375), *Bacillus cereus* (NRRL B-3711), *Proteus vulgaris* (NRRL B-123), *Salmonella typhimurium* (NRRL B-4420), *Staphylococcus aureus* (NRRL B-767), *Escherichia coli* (NRRL B-3704), *Candida albicans*, and *Candida glabrata* were investigated, and significant activity was observed.*

**Keywords** Antimicrobial activity; benzo[d]imidazole; benzo[d]oxazole; Fluorene

## INTRODUCTION

In general, bacterial pathogens may be classified as either Gram-positive or Gram-negative pathogens. Antibiotic compounds with effective activity against both Gram-positive and Gram-negative pathogens are generally regarded as having a broad spectrum of activity. The compounds presented here exhibit activity against both Gram-positive and Gram-negative pathogens.

Received June 7, 2006; accepted August 23, 2006.

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The international microbiological community continues to express serious concern in view of the alarming increase of resistance to commercially available antibiotics; this resistance reduces the range of possibilities of treatment of the different infectious processes.<sup>1-5</sup> Especially resistance against  $\beta$ -lactam antibiotics, macrolides, quinolones, and vancomycin is among the most important worldwide<sup>1,2,6-8]</sup> health problems. In particular, the increasing antibiotic resistance of Gram-positive bacteria is becoming a serious and important problem for human beings.<sup>9-12</sup>

Beside this, the lack of new antifungal drugs ascends proportionally to the increasing occurrence of serious infections caused by yeast and fungi, mainly in immunocompromised or in other way sensitive patients. Primary and opportunistic fungal infections continue to increase rapidly, and as a consequence of this situation, invasive fungal infections constitute a major cause of mortality for these patients. *Candida albicans* is one of the most common opportunistic fungi responsible for these kinds of infections. The current state of pharmacotherapy is briefly drawn out: most attention is given to newly developed active entities. Established agents do not satisfy the medical need completely; azoles are fungistatic and vulnerable to resistance, whereas polyenes cause serious host toxicity. Drugs in clinical development include modified azoles and a new class of echinocandins and pneumocandins.<sup>13-15</sup>

In order to overcome this rapid development of drug resistance, new agents should preferably have chemical characteristics that clearly differ from those of existing agents. In drug-designing programs, an essential component of the search for new leads is the synthesis of molecules, which are novel but still resemble known biologically active molecules by virtue of the presence of critical structural features. Certain small heterocyclic molecules act as highly functionalized scaffolds and are known pharmacophores of a number of biologically active and medically useful molecules.<sup>16,17</sup>

In the last few years, benzoxazole and the related heterocycle benzimidazole have been extensively studied for their antitumor, antiviral, and antibiotic activities as new non-nucleoside topoisomerase I poisons, HIV-1 reverse transcriptase inhibitors, and/or potent DNA gyrase inhibitors. The structural similarity of benzoxazole derivatives with nucleic bases, such as adenine and guanine, probably allows their easy interaction with biopolymers in living systems. Consequently, these compounds possess a wide range of biological activities.<sup>18</sup>

A 2-substituted benzoxazole derivative (L-697,661) was observed as a specific non-nucleoside reverse transcriptase inhibitor for Human Immunodeficiency Virus type 1 (HIV-1).<sup>19</sup>

The other series of 2-substituted benzoxazoles and benzimidazoles were synthesized and studied as topoisomerase I inhibitors.<sup>20</sup> In evaluating their cytotoxicity, these new topoisomerase I poisons also exhibited no significant cross-resistance against cell lines that express camptothecin-resistant topoisomerase I.

On the other hand, benzimidazole compounds have proven to be the most important group of fungicides with systemic activity and are well known for their pronounced ability to control a large number of fungal diseases.<sup>21</sup>

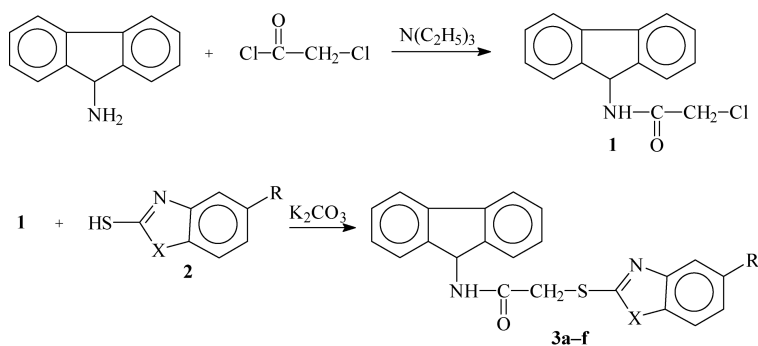
In the interest of what we previously discussed, we planned to synthesize 2-(benzo[d]oxazol/benzo[d]imidazol-2-ylthio)-*N*-(9*H*-fluoren-9-yl)acetamide that includes benzoxazoles or benzimidazoles to give a compact structure.

## RESULTS AND DISCUSSION

### Chemistry

In the present work, 6 new compounds (**3a–f**) were synthesized (Scheme 1). Structures of the obtained compounds were confirmed by their spectral data. In <sup>1</sup>H NMR spectrum of compounds, the S-CH<sub>2</sub> methylene protons appeared at 4.05–4.30 ppm as singlets. The fluorene C<sub>9</sub> proton was observed at 6.05–6.10 ppm as doublets. The signal of the NH–C=O proton was observed at 8.90–9.00 ppm as a doublet. All other aromatic and aliphatic protons were observed in the expected regions. All compounds gave satisfactory elemental analyses.

Mass spectra (FAB) of the compounds showed M+1 peaks in agreement with the respective molecular formula.



2,3	a	b	c	d	e	f
X	O	O	O	NH	NH	NH
R	H	Cl	CH <sub>3</sub>	H	Cl	CH <sub>3</sub>

SCHEME 1

## Microbiology

Minimum Inhibitory Controls (MICs) were recorded as the minimum concentration of compound, which inhibits the growth of the tested microorganisms. All of the compounds tested showed significant antibacterial and antifungal activity when compared with Chloramphenicol and Ketoconazole.

The antibacterial assessment revealed that the compounds possess significant activity. The MIC values were generally within the range 0.97–250  $\mu\text{g/mL}$  against all evaluated strains.

By comparing the MIC values of compounds **3** with that of chloramphenicol, all of the new derivatives were highly effective against *Bacillus cereus*.

Heterocycles **3** exhibited significant activities against *Micrococcus luteus* and *Proteus vulgaris*; especially **3d** showed strong activity, **3e** similar and **3b** and **3c** moderate activity against *M. luteus*. All of the compounds investigated showed moderate activity against *P. vulgaris* when compared with the reference agent.

On the other hand, heterocycles **3** exhibited comparable activities against *Staphylococcus aureus* and *Escherichia coli*. **3c** showed strong activity and the other compounds showed similar activity against *S. aureus*. **3c** and **3d** exhibited strong activity and the other compounds showed similar activity against *E. coli* with respect to the reference agent.

Fluorenes **3** were less active against and *Salmonella typhimurium*.

Most of the heterocycles were effective against *Candida glabrata* when compared with ketoconazole; in particular **3b** showed strong activity, **3d** showed similar activity, and the other compounds showed moderate activity.

Similar results were obtained for *C. albicans*: compound **3b** showed strong activity, **3d** showed similar activity and the other compounds moderate activity when compared with ketoconazole.

## EXPERIMENTAL

### Chemistry

All melting points were determined in open capillaries on a Gallenkamp apparatus (Weiss-Gallenkamp, Loughborough, United Kingdom) and are uncorrected. The purity of the compounds was routinely checked by TLC using silica gel 60G (Merck, Darmstadt, Germany). Spectroscopic data were recorded with the following instruments: IR; Shimadzu IR-435 spectrophotometer (Shimadzu, Tokyo, Japan);  $^1\text{H}$  NMR; Bruker

250 MHz spectrometer (Bruker, Billerica, Massachusetts, USA) in DMSO- $d_6$  using TMS as internal standard; and MS-FAB, VG Quattro Mass spectrometer (Agilent, Minnesota, USA). Elemental analyses were performed with a Leco CHNS-932 (LECO Corporation, Michigan, USA) instrument.

### 9-(Chloroacetylamino)fluorene (1)

9-(chloroacetylamino)fluorene (**1**) was prepared by reacting 9-aminofluorene with chloroacetyl chloride following the method described in the literature.<sup>22–24</sup>

### 2-(Benzo[d]oxazol/benzo[d]imidazol-2-ylthio)-N-(9H-fluoren-9-yl)acetamides (3a-f): General Procedure

A mixture of 9-(chloroacetylamino)fluorene (**1**) (0.01 mol), benzo[d]oxazol-benzo[d]imidazol-2-thiole (**2**) (0.01 mol) and  $K_2CO_3$  (0.01 mol) in acetone (50 mL) was refluxed for 6–10 h. After cooling to r.t., the solution was evaporated to dryness. The residue was washed with water (200 mL) and recrystallized from ethanol (Scheme 1).

Some characteristics of the synthesized compounds are shown in Table I.

### 2-(Benzo[d]oxazol-2-ylthio)-N-(9H-fluoren-9-yl)acetamide (3a)

IR (KBr),  $\nu$ : 3255, 1740, 1651, 1575  $cm^{-1}$ ;  $^1H$  NMR (DMSO- $d_6$ ): 4.30 (s, 2H), 6.05 (d,  $J = 8.3$  Hz, 1H), 7.30–7.70 (m, 10H), 7.90 (d,  $J = 7.9$  Hz, 2H), 9.00 (d,  $J = 8.3$  Hz, 1H); MS (FAB) ( $M+1$ ):  $m/z$  373; calcd. for  $C_{22}H_{16}N_2O_2S$ : C, 70.95; H, 4.33; N, 7.52. Found: C, 71.02; H, 4.34; N, 7.59.

### 2-[(5-Chlorobenzo[d]oxazol-2-yl)thio]-N-(9H-fluoren-9-yl)acetamide (3b)

IR (KBr),  $\nu$ : 3244, 1745, 1645, 1571  $cm^{-1}$ ;  $^1H$  NMR (DMSO- $d_6$ ): 4.25 (s, 2H), 6.00 (d,  $J = 8.3$  Hz, 1H), 7.25–7.70 (m, 9H), 7.80–7.90 (m, 2H), (d,  $J = 8.4$  Hz, 1H); MS (FAB) ( $M+1$ ):  $m/z$  407; calcd. for  $C_{22}H_{15}ClN_2O_2S$ : C, 64.94; H, 3.72; N, 6.88. Found: C, 65.05; H, 3.73; N, 7.05.

TABLE I Some Characteristics of Compounds 3

	Yield (%)	M.P. (°C)	Mol. formula	Mol. weight
<b>3a</b>	85	217	$C_{22}H_{16}N_2O_2S$	372.44
<b>3b</b>	80	210	$C_{22}H_{15}ClN_2O_2S$	406.88
<b>3c</b>	85	222	$C_{23}H_{18}N_2O_2S$	386.47
<b>3d</b>	80	246	$C_{22}H_{17}N_3OS$	371.45
<b>3e</b>	79	275	$C_{22}H_{16}ClN_3OS$	405.90
<b>3f</b>	78	254	$C_{23}H_{19}N_3OS$	385.48

**2-[(5-Methylbenzo[d]oxazol-2-yl)thio]-N-(9H-fluoren-9-yl)acetamide (3c)**

IR (KBr),  $\nu$ : 3254, 1743, 1640, 1568  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{DMSO}-d_6$ ): 2.45 (s, 3H), 4.20 (s, 2H), 6.00 (d,  $J = 8.2$  Hz, 1H), 7.10 - 7.50 (m, 9H), 7.85 (d,  $J = 7.9$  Hz, 2H), 8.90 (d,  $J = 8.4$  Hz, 1H); MS (FAB) ( $M+1$ ):  $m/z$  387; Calcd. for  $\text{C}_{23}\text{H}_{18}\text{N}_2\text{O}_2\text{S}$ , C, 71.48; H, 4.69; N, 7.25. Found: C, 71.35; H, 4.61; N, 7.08.

**2-(Benzo[d]imidazol-2-ylthio)-N-(9H-fluoren-9-yl)acetamide (3d)**

IR (KBr),  $\nu$ : 3264, 1740, 1645, 1572  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{DMSO}-d_6$ ): 4.15 (s, 2H), 6.00 (d,  $J = 8.2$  Hz, 1H), 7.15–7.50 (m, 10H), 7.80 (d,  $J = 7.5$  Hz, 2H), 8.90 (d,  $J = 8.4$  Hz, 1H), 12.60 (br., 1H,); MS (FAB) ( $M+1$ ):  $m/z$  372; calcd. for  $\text{C}_{22}\text{H}_{17}\text{N}_3\text{OS}$ : C, 71.14; H, 4.61; N, 11.31. Found: C, 71.32; H, 4.70; N, 11.07.

**2-[(5-Chlorobenzo[d]imidazol-2-yl)thio]-N-(9H-fluoren-9-yl)acetamide (3e)**

IR (KBr),  $\nu$ : 3252, 1742, 1650, 1570  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{DMSO}-d_6$ ): 4.20 (s, 2H), 6.00 (d,  $J = 8.3$  Hz, 1H), 7.10–7.45 (m, 9H), 7.85 (d,  $J = 7.5$  Hz, 2H), 8.95 (d,  $J = 8.3$  Hz, 1H), 12.70 (br., 1H,); MS (FAB) ( $M+1$ ):  $m/z$  406; calcd. for  $\text{C}_{22}\text{H}_{16}\text{ClN}_3\text{OS}$ : C, 65.10; H, 3.97; N, 10.35. Found: C, 64.82; H, 4.03; N, 10.58.

**2-[(5-Methylbenzo[d]imidazol-2-yl)thio]-N-(9H-fluoren-9-yl)acetamide (3f)**

IR (KBr),  $\nu$ : 3244, 1735, 1645, 1569  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{DMSO}-d_6$ ): 2.35 (s, 3H), 4.05 (s, 2H), 6.00 (d,  $J = 8.2$  Hz, 1H), 6.90–7.45 (m, 9H), 7.85 (d,  $J = 7.4$  Hz, 2H), 8.90 (d,  $J = 8.2$  Hz, 1H), 12.45 (br., 1H); MS (FAB) ( $M+1$ ):  $m/z$  386; calcd. for  $\text{C}_{23}\text{H}_{19}\text{N}_3\text{OS}$ : C, 71.66; H, 4.97; N, 10.90. Found: C, 71.81; H, 4.96; N, 10.77.

## Microbiology

The antimicrobial activities of compounds **3** were tested using the microbroth dilution method.<sup>25</sup> Tested microorganism strains were *M. luteus* (NRRL B-4375), *B. cereus* (NRRL B-3711), *P. vulgaris* (NRRL B-123), *S. typhimurium* (NRRL B-4420), *S. aureus* (NRRL B-767), *E. coli* (NRRL B-3704), *C. albicans*, and *C. glabrata* (isolates obtained from Osmangazi University, Faculty of Medicine, Eskisehir, Turkey). Microbroth dilution-susceptibility assay was used for antimicrobial



TABLE II Antimicrobial Activities of Compounds 3 ( $\mu\text{g/mL}$ )

	A	B	C	D	E	F	G	H
<b>3a</b>	31.25	7.8	3.9	31.25	62.5	31.25	125	125
<b>3b</b>	7.8	3.9	3.9	62.5	62.5	62.5	31.25	31.25
<b>3c</b>	3.9	7.8	7.8	31.25	31.25	31.25	125	125
<b>3d</b>	0.97	15.6	7.8	62.5	62.5	31.25	62.5	62.5
<b>3e</b>	1.95	15.6	1.95	62.5	62.5	62.5	250	250
<b>3f</b>	31.25	15.6	3.9	62.5	62.5	62.5	125	125
Reference 1	1.95	125	0.97	0.97	62.5	62.5	—	—
Reference 2	—	—	—	—	—	—	62.5	62.5

**Reference 1:** Chloramphenicol; **Reference 2:** Ketoconazole.

**A:** *M. luteus* (NRRL B-4375), **B:** *B. cereus* (NRRL B-3711), **C:** *P. vulgaris* (NRRL B-123), **D:** *S. typhimurium* (NRRL B-4420), **E:** *S. aureus* (NRRL B-767), **F:** *E. coli* (NRRL B-3704), **G:** *C. albicans* (isolates obtained from Osmangazi University), **H:** *C. glabrata* (isolates obtained from Osmangazi University).

evaluation of the compounds. Stock solutions of the samples were prepared in dimethylsulfoxide. Dilution series using sterile distilled water were prepared from 4 mg/mL to 0.007 mg/mL in micro-test tubes that were transferred to 96-well microtiter plates. Overnight-grown bacterial and *C. albicans* suspensions in double-strength Mueller–Hinton broth were standardized to  $10^8$  CFU/mL using McFarland No. 0.5 standard solution. hundred microliter of each microorganism suspension was then added into the wells. The last well-chain without a microorganism was used as a negative control. Sterile distilled water and the medium served as a positive growth control. After incubation at  $37^\circ\text{C}$  for 18–24 h, the first well without turbidity was determined as the MIC. Chloramphenicol was used as standard antibacterial agent, whereas ketoconazole was used as an antifungal agent. Observed data on the antimicrobial activity of the compounds and control drugs are given in Table II.

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